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Thomas, Rhian; Gough, Rachel; Freeman, Christopher

Ecological Engineering

Published: 01/09/2017

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):

Thomas, R., Gough, R., & Freeman, C. (2017). Linear alkylbenzene sulfonate (LAS) removal in constructed wetlands: The role of plants in the treatment of a typical pharmaceutical and personal care product. *Ecological Engineering*, 106(part A), 415-422.

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Linear alkylbenzene sulfonate (LAS) removal in constructed wetlands: The role of plants

Rhian Thomas^{1*}, Rachel Gough¹, Christopher Freeman¹

¹ School of Biological Sciences, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK

*Corresponding author: rh_tom@hotmail.com.

Abstract

Linear alkylbenzene sulphonate (LAS) is a major anionic surfactant used in detergents worldwide and as such is a ubiquitous constituent of domestic and municipal wastewaters. Increasingly, constructed wetlands are being employed as a low cost and sustainable alternative to traditional wastewater treatment processes. Plants are known to play a vital role both directly and indirectly in the removal of contaminants in wastewater treatment constructed wetlands. However, relatively little research has been conducted into the manipulation of the plant component in order to optimise constructed wetland performance. Furthermore, little is known about the role of plants in the removal of specific contaminants including LAS. The present study investigated the effects of plant biomass and plant species on LAS removal in a series of experimental subsurface flow wetlands. Our results confirm that the presence of vegetation enhances LAS removal, with higher biomass systems associated with higher LAS removal rates. Differences in LAS removal were also observed between different plant species, although these were not found to be statistically significant.

Key words: biomass; constructed wetlands; enzymes; linear alkylbenzene sulphonate (LAS); plant species; wastewater.

Introduction

Constructed wetlands are designed to mimic the biogeochemical characteristics and functions of a natural wetland under more controlled conditions. On account of their natural nutrient cycling capacity, the application of constructed wetlands for sewage treatment has been popular, with over 50,000 systems reported to be in operation in Europe (Wu *et al.* 2015), usually in a secondary or tertiary capacity. The effectiveness of the wetland is based on various complex physical, chemical and biological processes occurring in parallel between the substrate, plants and microorganisms. Surveys on the removal of various pollutants in constructed wetlands have been conducted globally, e.g. 5-day Biochemical Oxygen Demand (BOD₅) (Sankararajan *et al.* 2017), phosphate (Ramasahayam *et al.* 2014), nitrate (Wu *et al.* 2014) and metals (Šíma *et al.* 2016). To date however, relatively few studies have considered the role of constructed wetlands in the removal of surfactants.

Surfactants are surface-active compounds that consist of both polar and non-polar parts (Swisher 1987). These compounds are widely used in detergents due to their unique surface-active properties (Swisher 1987) and are therefore a major component of urban wastewaters. Linear alkylbenzene sulphonate (LAS) is a major anionic surfactant used in detergents worldwide due to its effectiveness, cost/performance ratio, versatility and environmental safety record (de Wolfe & Feijtel 1998). It is the most widely used synthetic anionic surfactant and is therefore an omnipresent water contaminant (Vymazal 2014). It can also be used as an indicator of the presence of other pharmaceuticals and personal care products (PPCPs) in surface waters (Nakada *et al.* 2008). The surfactant was introduced in the 1960s as a replacement for slowly degradable alkylbenzene sulphonate (ABS). Foaming problems in sewage treatment plants, rivers and lakes mainly due to ABS are well documented (Jensen

1999). Since the foaming problems of the 1960s, regulations have been introduced stipulating that surfactants released into the environment must exhibit high biodegradation capacities. Only limited research has been conducted into the fate of LAS and other surfactants in wetlands; Inaba *et al.* (1988) assessed LAS removal in a large-scale natural wetland system in Japan and reported seasonal variation in LAS removal due to temperature-driven changes in biodegradation by bacteria and/or adsorption on sediment particles. Longer alkyl chain homologues are reported to be removed to a greater extent than shorter alkyl chains (Billore *et al.* 2002; Thomas *et al.* 2003). Research has also shown that shallower beds, where more oxygenated conditions occur, are associated with the highest rates of LAS degradation (Huang *et al.* 2004).

Plants are known to play a key role in various physical, chemical and biological processes in a wetland. For example, they serve to stabilize the bed surface, insulate against freezing and frost through litter production, prevent clogging, shield algae from incoming solar radiation, adsorb and store nutrients, and prevent channelled flow (Brix 1997, Kadlec & Knight 1996). However, there remains a lack of knowledge and quantitative data on the role of plants in wastewater treatment with information mainly centered on nutrient rather than pollutant removal. Debate has arisen over the necessity of plants and adverse impacts reported in some cases e.g. acid mine drainage treatment (King & Garey 1999). However, the impact plants have will depend on the individual constructed wetlands in terms of their design, loading, type of treatment and environmental conditions (Vymazal 2009, Carballeira *et al.* 2016). Published research suggests that greater LAS removal occurs when plants are present (Federle & Schwab 1989). The present study aims to develop a more comprehensive understanding of the role of plants in LAS removal by investigating the influence of plant biomass and plant species on LAS removal in constructed wetlands.

This paper presents the results of a 6-month field based study comparing planted and unplanted systems in mesocosm experimental subsurface flow wetlands. Plant biomass (zero, low and high) effects were assessed at the same field site over a 15-day experiment. Finally, a microcosm laboratory experiment was conducted to assess the effect of plant species on LAS removal. Substrate enzyme activity was also compared between treatments.

Methods

Experiment 1: LAS removal in planted and unplanted mesocosm systems

Eight identical sub-surface flow wetland mesocosms (Figure 1) were constructed at an outdoor site in Abergwyngregyn, north Wales (grid ref. SH 655736). The mesocosms measured 1.95 m (l) x 0.65 m (w) x 0.4 m (d) and were filled with gravel (approximately 5-10 mm diameter) to a depth of 0.38 m. Four of the mesocosms were planted with *Phragmites australis* at a density of 4 plants per m² and the remaining 4 mesocosms were left unplanted. The design incorporated 2 large storage tanks, each connected *via* smaller storage tanks to 4 mesocosms arranged in parallel. Flow was controlled using a tap and ball cock valve system. Inflow rates of 35 L day⁻¹ of 5 mg L⁻¹ LAS in distilled water were applied on a continuous basis to mimic full-scale operational constructed wetlands flow and LAS loading at a typical wastewater treatment plant. The theoretical hydraulic residence time (nHRT) was 13.8 days. Outflow water from each mesocosm was sampled on a monthly basis over a 6-month period (September 2000 to February 2001). The addition of LAS-spiked water began at the start of September 2000 with the first samples taken at the end of the month. LAS concentration measurements were

conducted on outflow water. Enzyme (phosphatase, β -glucosidase and sulphatase) measurements were conducted on 5 replicate gravel grab samples (150 cm³/ 5-10 cm depth) collected monthly from each mesocosm.

Experiment 2: Effect of plant biomass on LAS removal

The mesocosms described in experiment 1 were also used to investigate the effect of plant biomass (zero, low and high) on LAS removal. Artificial sewage (see Table 1) containing 10 mg L⁻¹ LAS (simulating normal – high LAS loading) was loaded onto the mesocosms continuously over a 15 day period (6-20th June 2001) at a rate of 35 L day⁻¹ (nHRT = 13.8 days). Sampling of outflow water began 1 day after the start of the treatment, with subsequent samples collected every 2 days. LAS concentration measurements were conducted on outflow water. As with experiment 1, gravel substrate samples (150 cm³) were also collected for enzyme (phosphatase, β -glucosidase and sulphatase) analyses. KBr (1.5 mg L⁻¹ Br⁻) was added to the water supply tank at the start of the experiment, as a chemical tracer to assess the hydraulic retention time.

Experiment 3: Effect of plant species on LAS removal

Small-scale replicate wetland microcosms were built to compare the effect of 5 different plant species on LAS removal. The microcosms were constructed from transparent plastic beakers (11.5 cm diameter x 13 cm depth) filled with gravel substrate and planted with a single specimen (*Phragmites australis*, *Typha latifolia*, *Salix viminalis*, *Iris* and *Juncus effusus*). Unplanted microcosms acted as a control. The microcosms (4 replicates per treatment) were stored in a temperature-controlled room maintained at 12°C. 350 mL of artificial sewage containing 10 mg L⁻¹ LAS was added to each microcosm at the beginning of the experiment. Sampling of water began 1 day after the addition of the sewage solution and continued on a daily basis until day 4, after which samples were collected every 4 days. Filters (cut off 2.5 mL Plastipak™ syringes packed with glass wool) were inserted as a sample port in the top of each microcosm. LAS concentration measurements were conducted on collected water.

Determination of LAS concentration

LAS Analytical Procedure

Quantification of LAS was based on the procedure developed by Matthijs & De Henau (1987), but modified slightly to improve selectivity. Prior to analysis, samples were filtered through a 0.2 μ m Whatman membrane filter. Solid Phase Extraction (SPE) was used to isolate and concentrate the LAS in the aqueous samples before HPLC analyses. Each sample was initially passed through a Hypersep C18 SPE column and then eluted with methanol onto a Hypersep SAX Anion Exchange SPE column (both Thermo Fisher Scientific, Waltham MA, US). LAS was then eluted into a glass vial with 3 mL of CH₃OH:HCl solution (80:20) and evaporated to dryness at 75°C under a gentle stream of nitrogen. The samples were stored in a dry state at <4°C before analyses. In order to minimise contamination all glassware was washed in methanol then conditioned with LAS solution for 24 h prior to use to reduce loss of surfactant to the glass surface.

HPLC Analyses

Separation of LAS homologues was achieved by reversed phase separation using a Dionex DX-300 HPLC system (Thermo Fisher Scientific, Waltham MA, US) equipped with a μ Bondclone C18 analytical column (Phenomenex, Torrance CA, US). Measurement was using a LS50 fluorescence spectrometer (PerkinElmer, Waltham MA, US) (excitation λ = 232 nm; emission λ = 290 nm; slit width = 10 nm). The mobile phase was a 22:78 distilled water:methanol solution containing sodium perchlorate buffer (0.0875 M) with the flow rate set to 2 mL min⁻¹. Calibration standards were Nansa HS 80/S alkylbenzene sulfonic acids containing C₁₀-C₁₃ LAS homologues (with alkyl chain distributions of C₁₀ 15.8%; C₁₁ 41.5%; C₁₂ 30.1%; C₁₃ 12.5%). LAS concentrations were derived by addition of the C₁₀-C₁₃ LAS homologue concentrations.

Enzyme assays

Activities of three hydrolytic enzymes (β -glucosidase, sulphatase and phosphatase) were determined in 5 replicate gravel samples from each mesocosm using fluorogenic methylumbelliferyl (MUF) substrates (Freeman *et al.* 1995). 2 mL of cellosolve (ethylene glycol monoethyl ether) was used to pre-dissolve all MUF substrates for each assay as substrates have minimal solubility in pure water. Cellosolve does not affect enzyme activity (Hoppe, 1983).

In a plastic stomacher bag, 7 mL of MUF substrate was added to 1 g of gravel sample, homogenised using a Seward Stomacher 80 Laboratory Blender and incubated at field temperature for 1 h. The reaction was terminated by centrifuging the mixture at 10,000 rpm for 5 min. 0.5 mL of supernatant was then added to 2.5 mL of deionised water and fluorescence determined with a LS50 fluorescence spectrometer (PerkinElmer, Waltham MA, US) (excitation λ = 330 nm; emission λ = 450 nm; slit width = 2.5 cm). Calibration curves were constructed using 0-100 μ M MUF-free acid solution and assayed as above.

Determination of KBr tracer

A Dionex DX-120 Ion Chromatograph equipped with an IonPac AS4A anion analytical column was used to measure the concentration of bromide. The eluent was 1.7 mM Na₂HCO₃/1.8 mM Na₂CO₃. The column was calibrated using standard Dionex solutions and a flow rate of 1 mL min⁻¹ was used.

Statistical Analysis of Results

Statistical analyses were conducted using Minitab™ version 13.1 (Minitab Inc. 2000). Differences between planted and unplanted treatments were assessed *via* paired t-tests. For differences between more than two treatments (species and biomass), repeated measures ANOVA tests were applied. For significant ANOVA results, the Tukey post-hoc test was used to identify where significant differences between groups lay.

Results

Planted vs. unplanted mesocosms

LAS removal

High LAS degradation was observed from the start of the experiment and increased with time (Figure 2). Outflow water LAS concentration in the unplanted mesocosms (mean 0.05 mg L^{-1}) was consistently higher than in the planted mesocosms (mean 0.02 mg L^{-1}). This difference was found to be statistically significant ($p < 0.01$). However, high LAS removal rates (>95%) were observed in both systems throughout the course of the experiment.

Enzyme activity

Enzyme activity was significantly higher for phosphatase, than β -glucosidase and sulphatase, by a minimum of a 2-fold factor in the planted ($F = 6.04$, $p < 0.01$) and unplanted ($F = 12.79$, $p < 0.001$) mesocosms (Figure 3). Unplanted systems exhibited higher mean enzyme activity in comparison to planted microcosms but this was only significant for phosphatase ($p < 0.05$). Both phosphatase and β -glucosidase activity decreased from the initial activity measured in September, especially for the unplanted systems (Figure 3a and 3b). In contrast, an increase in sulphatase activity from initial levels after LAS addition was observed (Figure 3c).

Effect of plant biomass on LAS removal

LAS

High LAS removal (>95%) was observed in all treatments with LAS concentrations increasing initially, and then decreasing in the last 7 days (Figure 4). Mean LAS removal rates varied as follows: high-biomass (0.08 mg L^{-1})>low-biomass (0.36 mg L^{-1})>unplanted (0.45 mg L^{-1}). Statistical analysis identified significant differences in LAS removal rates between treatments ($F = 8.26$, $p < 0.01$). However, the post-hoc test revealed no significant differences between the unplanted and low biomass planted treatments.

Enzyme activity

Highest activity in all treatments was observed for phosphatase, followed by β -glucosidase and sulphatase, respectively (Figure 5). No statistically significant correlations were identified between enzymes in different treatments, except for phosphatase ($p < 0.05$), reflecting the large fluctuations in activity measured. The only statistically significant differences between planted and unplanted treatments was observed for sulphatase ($F = 15.192$, $p < 0.001$). Compared with the levels reported for experiment 1, approximately 4-fold higher phosphatase and β -glucosidase activity was observed. However, in contrast, lower sulphatase activity was observed. This was more prominent in the planted (low-biomass -30%, high-biomass -60%) than unplanted (-20%) mesocosms.

Tracer study

Figure 6 shows the tracer study results which provide an indication of the diffusion rate. Faster recovery was observed in the unplanted, compared with the low and high-biomass mesocosms, respectively. The control/high-biomass comparison was statistically significant ($F = 7.756$, $p < 0.01$). A slower initial diffusion rate was exhibited for the planted treatments in comparison to unplanted.

Effect of plant species on LAS removal

LAS

The mean concentrations measured after 12 days in the six treatments were, in descending order, the gravel control (1.83 mg L⁻¹), *Typha* (0.23 mg L⁻¹), *Iris* (0.12 mg L⁻¹), *Juncus* (0.10 mg L⁻¹), *Salix* (0.09 mg L⁻¹) and *Phragmites* (0.08 mg L⁻¹) (Figure 7). A marked and statistically significant difference in LAS concentration was observed between the unplanted and each of the planted treatments ($p < 0.001$). No significant difference was identified between planted treatments.

Discussion

LAS removal in planted and unplanted systems

LAS

The high LAS removal rates observed both in the planted and unplanted systems suggest that constructed wetlands have high potential for LAS degradation under optimal conditions. However, significantly higher LAS outflow concentration in the unplanted system suggests that the presence of vegetation enhances LAS treatment efficiency. This is consistent with the findings of previous studies which report higher removal of organic matter (Allen *et al.* 2002), nutrients (Heritage *et al.* 1995), heavy metals (Doyle & Otte 1997) and ammonia (Sikora *et al.* 1995) in planted systems. Federle & Schwab (1989) reported a higher rate of LAS mineralization with microbiota associated with aqueous plants in the rhizosphere than in nearby root-free sediment. This may be explained by several possible plant mechanisms facilitating microbial activity, including rhizosphere oxygen release, rhizosphere and root attachment sites for bacterial growth, DOC root release enhancing bacterial activity and plant uptake (Brix 1994, Brix 1997). The high removal efficiency observed in the unplanted systems suggests that related physical processes such as adsorption or formation of biofilms on the gravel surface contribute significantly to LAS removal. Indeed Fountoulakis *et al.* (2009) report that adsorption onto media is the main mechanism for LAS removal in their pilot constructed wetland systems.

The observed general decline in LAS concentration with time could be explained by the acclimatisation of the microcosm bacterial community to the surfactant. Microbial communities acclimated by pre-exposure to the surfactant are enriched in organisms capable of degrading the compound, resulting shifts in community structure with increasing dominance in populations of these organisms (Federle & Pastwa 1988). Previous adaptation accelerated by initial LAS degradation is reported (Larson & Payne 1981, Palmisno *et al.* 1991, Federle & Pastwa 1988, Branner *et al.* 1999, Jensen 1999). Brown (1995) suggests that the bacterial population can increase its capacity to degrade surfactants by, for example, population growth potentially increasing the number of degraders, an increase in the amount of enzyme per cell biosynthesised, or random genetic mutations increasing biodegradation activity or creating new activity. Terzic *et al.* (1992) reported that the composition of a mixed bacterial culture rather than total number of bacteria determined biodegradation efficiency. Larson & Payne (1981) reported a shorter half-life with a 10-fold faster rate for degradation tests with river sediment collected closest to the vicinity of the effluent from a sewage treatment plant, similarly suggesting adaptation of communities receiving higher LAS concentrations. Shimp *et al.* (1989) reported greater

numbers of LAS degrading microorganisms, reduced lag period and higher degradation in water samples collected from an effluent exposed site compared with a pristine control site.

Enzyme activity

Measurement of enzyme activity showed that greatest activity occurred for phosphatase, confirming the findings of previous studies (Freeman *et al.* 1995, Chappell & Goulder 1992). The low enzyme activity observed overall may reflect the low nutrient and organic carbon availability in the system. The decrease in phosphatase and β -glucosidase activity over time, especially for the unplanted systems, may suggest toxicity or lack of suitable substrate and nutrients.

Surprisingly, planted systems exhibited lower enzyme activity than unplanted systems. Previous studies have shown higher phosphatase activity in planted compared with unplanted systems (Khan 1970, Neal 1973, Kiss *et al.* 1974 as quoted in Speir & Ross 1978). This effect may be indirect and caused by changes in organic matter and microbial populations brought about by the plants with highest activity when growth was most intensive (Speir & Ross 1978).

Effect of plant biomass on LAS removal

LAS

The occurrence of highest LAS removal in the high biomass systems suggests that wetlands with a high plant biomass ratio will promote LAS removal to a greater degree than comparative low plant biomass ratio wetlands. Knaebel & Vestal (1992) reported that the amount of above ground plant biomass correlated positively with the initial rates of mineralization. Wiessner *et al.* (2002) reported that the total size of the root system did not significantly affect the amount of oxygen root release but was governed by the size of the above ground biomass.

KBr was chosen as a tracer in this study due to its stability and ease of analysis (Tanner *et al.* 1998). Tanner *et al.* (1998) reported similar curves in a rain-free tracer study using bromide to those shown here. The results suggest slower diffusion rates in the planted mesocosms, especially in the high biomass treatment. The slower the diffusion rate, the greater the contact time between microorganisms and LAS within the wetland, promoting greater biodegradation. Greater removal has been reported in wetland systems with longer retention times. For example, greater removal of TN, TP and COD is reported with a 5 day retention time compared with a 2.5 day retention time (Breen 1997). Tanner (1994) reported a positive correlation between removal and retention time. Fisher (1990) and Marstener *et al.* (1996) found that plant roots markedly affected the hydraulic flow profiles in the upper layers of gravel wetlands in comparison to unplanted controls (as quoted in Tanner *et al.* 1998). On the other hand, the faster diffusion rate for the unplanted control may suggest potential short-circuiting. Gravel substrate can cause problems with non-uniform and short-circuiting flow of wastewater through the wetland (King *et al.* 1997). Factors, such as clogging by solid particles, can lead to preferential flow paths occurring. However, channelling within planted wetlands has also been reported (Bavour *et al.* 1988 as quoted in King *et al.* 1997).

Enzyme activity

No significant difference between treatments in enzyme activity was observed, except for sulphatase which was highest in the unplanted mesocosms. This contradicts the hypothesis that planted

mesocosms would exhibit higher activities. A comparison of the activity of different enzymes (phosphatase> β -glucosidase>sulphatase) supports findings reported previously (Freeman *et al.* 1995). Higher phosphatase and β -glucosidase activities compared with the planted/unplanted experiment reflect the higher nutrient input with levels more reflective of operational wetlands. The stimulated activity may also reflect the warmer temperature (Kang & Freeman 1998). Higher activity associated with plant growth mechanisms reported elsewhere (Speir & Ross 1978) is unlikely as the increase was observed in both planted and unplanted mesocosms.

Lower sulphatase activity compared with the planted/unplanted experiment may suggest inhibition of this enzyme. Inhibition may be due to specific effects on microbial growth and subsequent enzyme synthesis or possible modification of the active site of the enzyme protein (Dinesh *et al.* 1995). Possible inhibition of enzyme activity and subsequent nutrient cycling by LAS is suggested elsewhere (Jensen 1999). The greater reduction observed in the planted compared with the unplanted mesocosms may suggest plant mechanisms enhancing the inhibitory effect. However, no further conclusions may be drawn from the data.

Effect of plant species on LAS removal

LAS

The high LAS removal rates observed in this laboratory-scale experiment (>98% in planted systems) again highlights the potential for high LAS removal in constructed wetland systems. This study also confirms greater LAS removal in planted treatments in comparison to the unplanted gravel control. Though small in an operational context, the difference between planted systems in terms of net percentage removal were significant.

Variations in treatment by different plant species have been reported previously (Allen *et al.* 2002, Zhu & Sikora 1995) and several studies support the order of treatment efficiency reported here. For example, greater removal by *Phragmites* than *Typha* for ammonia and BOD (Gersberg *et al.* 1986), ammonium and nitrate (Zhu & Sikora 1995) and TN and TP (House *et al.* 1994) has been reported. Gersberg *et al.* (1986) attributed this result to the enhanced ability of *Phragmites* to pass oxygen into the root-zone. However, Burgoon *et al.* (1990) found *Typha* removed a significantly larger percentage of BOD₅ and total phosphate than *Phragmites* and Coleman *et al.* (2001) reported that *Typha* outperformed *Juncus* in wastewater treatment.

Although this experiment was conducted under controlled conditions, it is recognised that the effect of environmental conditions on treatment performance may vary between plant species. For example, *Phragmites* has an optimal pH of 2-8, *Typha* of pH 4-10 and *Juncus* of pH 5-7.5 (Reed *et al.* 1995). Plant biomass will also influence treatment efficiency. *Phragmites* is reported to have much deeper root penetration in gravel than *Typha* (Reed *et al.* 1995).

The effect of using a mixture of species on treatment efficiency has also been investigated with some evidence of improved performance using mixtures compared with monocultures (Coleman *et al.* 2001). However, the issue of competition between species is also important since this can cause a shift in species assemblage. Coleman *et al.* (2001) found that *Typha* was the superior competitor in plant mixture mesocosms, whereas *Juncus* is unlikely to be competitive (Tanner 1996).

Conclusion

This study investigated the importance of plants in LAS removal in small-scale experimental wetlands. The effect of plant biomass and species was assessed and a comparison of substrate enzyme activity conducted between treatments. All of the experiments confirmed that the presence of plants significantly enhanced LAS removal. No clear relationship between the presence/absence of plants and analysed substrate enzyme activity was evident from the data. However, for the longer-term experiment (experiment 1, lasting 6 months) and the plant species experiment (experiment 3) LAS removal increased with time, possibly due to acclimatisation of the microcosm bacterial community to the surfactant. Further research is necessary to develop a more comprehensive understanding of the mechanisms involved in the degradation of LAS and the role of plants therein. In particular, research should focus on the relative importance of biological and physical processes as well as their interaction, and the impact of environmental parameters such as temperature, pH and oxygen availability.

Acknowledgements

We would like to acknowledge the advice and assistance provided by Dr Kay Fox and Dr Naheed Rehman at Unilever.

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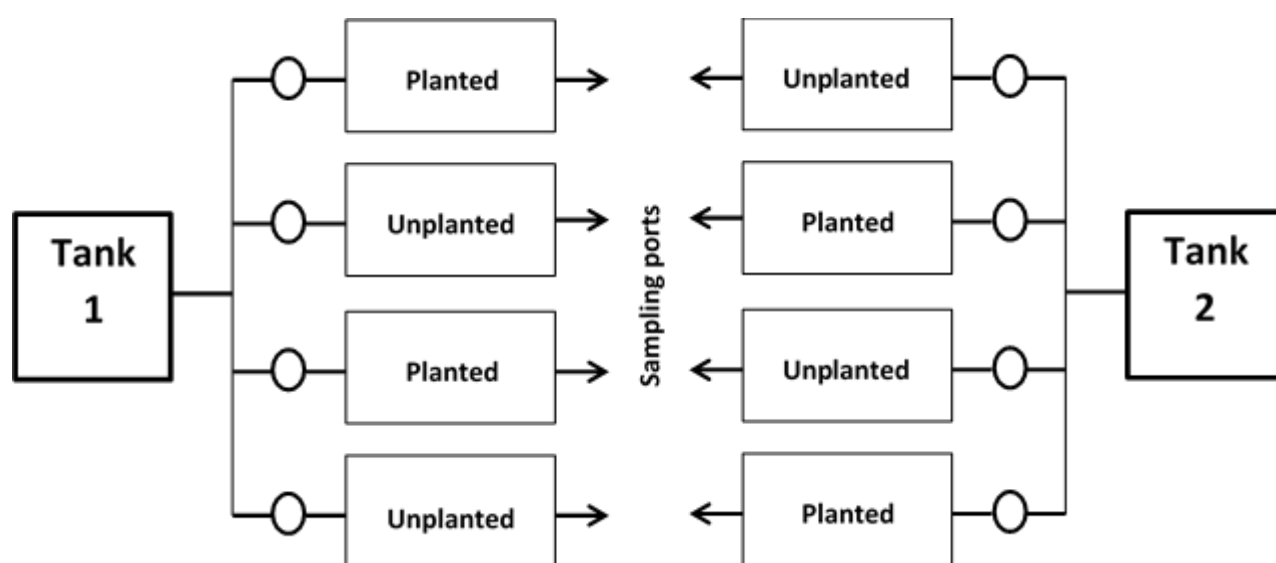


Figure 1. Constructed wetland mesocosm setup used for experiments 1 and 2

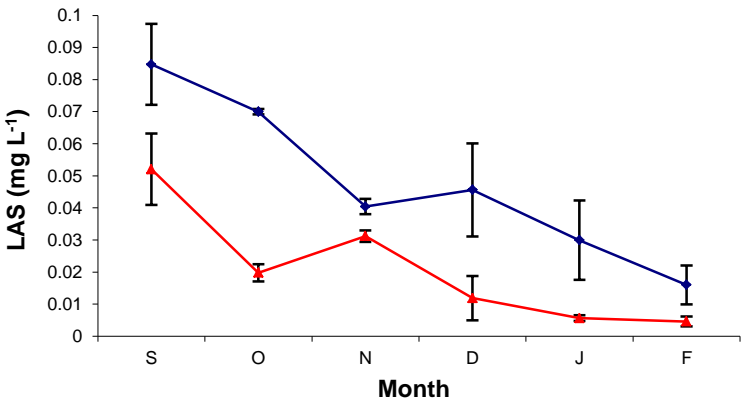
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Table 1. Recipe for artificial sewage solution

Ingredient	Concentration (mg L ⁻¹)	Acid	Concentration (mg L ⁻¹)
Peptone	70	Sodium acetate	136
Urea	25	Sodium propionate	28
Sucrose	35	Sodium butyrate	12
Soluble starch	35	Sodium benzoate	100
Ammonium sulphate	140	Sodium citrate	44
Mixed acids	105		
Potassium hydrogen phosphate	28		
Ferrous ammonium sulphate	21		
Trace metals solution	1 mL		

Metal	Concentration (mg L ⁻¹)
CuCl ₂ .2H ₂ O	0.25
Co(NO ₃).6H ₂ O	0.25
Na ₂ B ₄ O ₇ .10H ₂ O	0.25
ZnCl ₂ .2H ₂ O	0.25
MnSO ₄ .H ₂ O	1.00
K ₂ Mo ₄	0.25
NH ₄ VO ₃	0.10

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461 Figure 2. Outflow LAS concentrations in planted (red) and unplanted (blue) mesocosms between
462 September and February. Error bars represent the standard error of the mean ($n = 4$).

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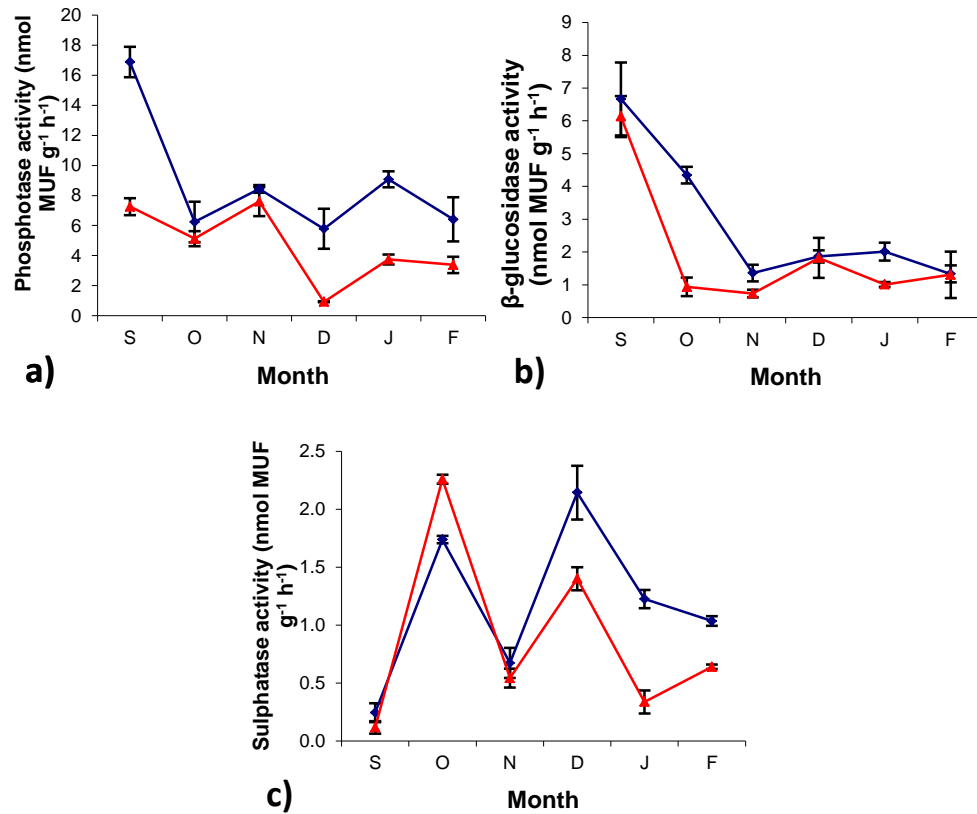


Figure 3. Enzyme activity in planted (red) and unplanted (blue) mesocosms between September and February showing phosphatase (a), β-glucosidase (b) and sulphatase (c). Error bars represent the standard error of the mean ($n = 4$).

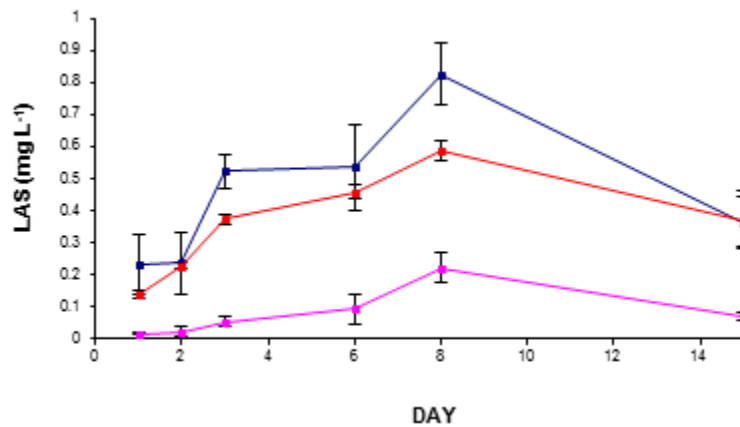


Figure 4. Outflow LAS concentrations for high density (pink), low density (red) and control (blue) plant biomass treatments. Error bars represent the standard error of the mean ($n = 4$).

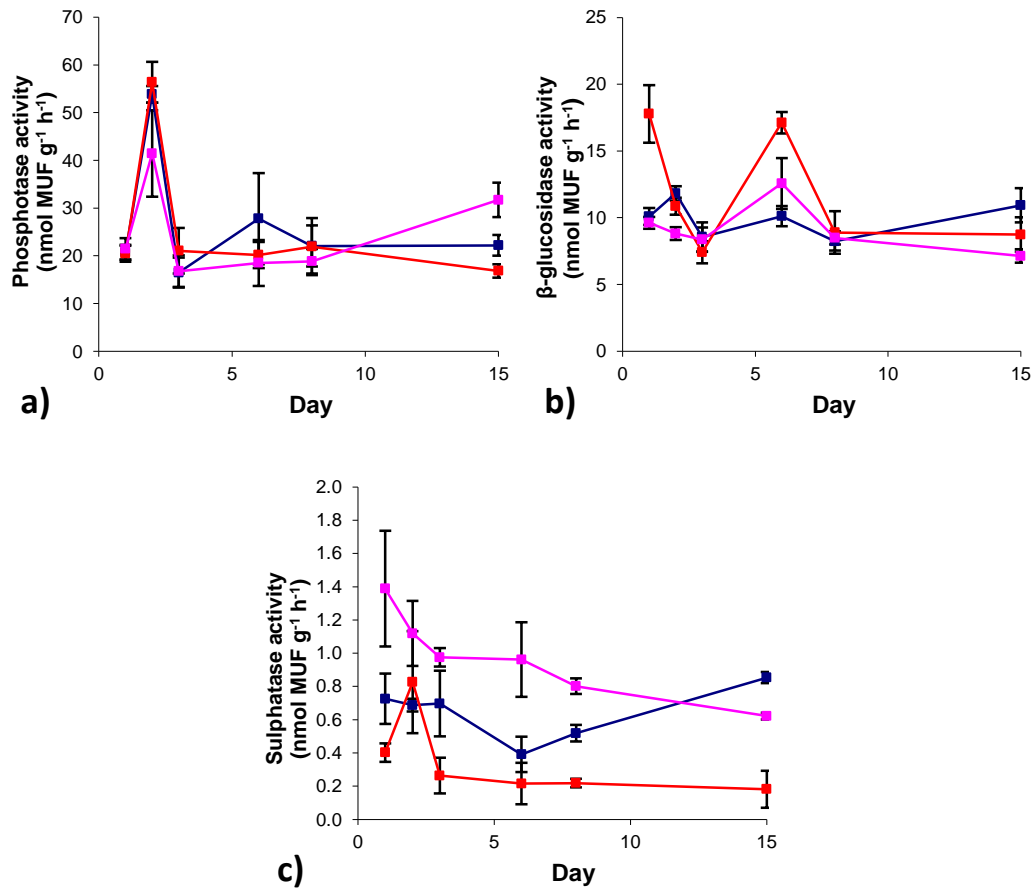


Figure 5. Enzyme activity in high density (pink), low density (red) and control (blue) plant biomass treatments showing phosphatase (a), β-glucosidase (b) and sulphatase (c). Error bars represent the standard error of the mean ($n = 4$).

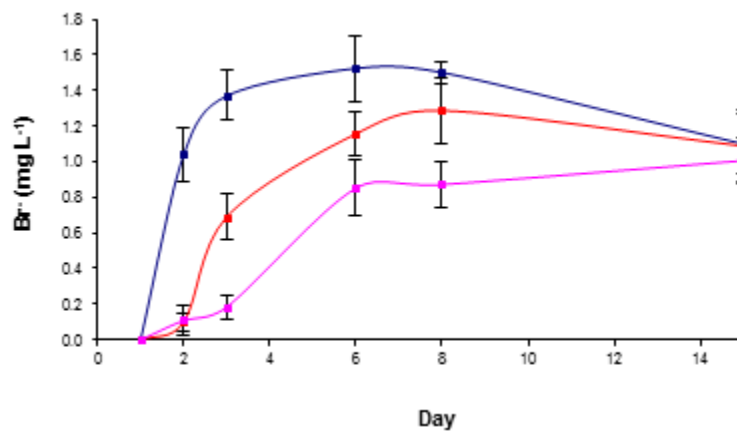


Figure 6. Br⁻ tracer study results showing Br⁻ concentration for the high density (pink), low density (red) and control (blue) plant biomass treatments. Error bars represent the standard error of the mean ($n = 4$).

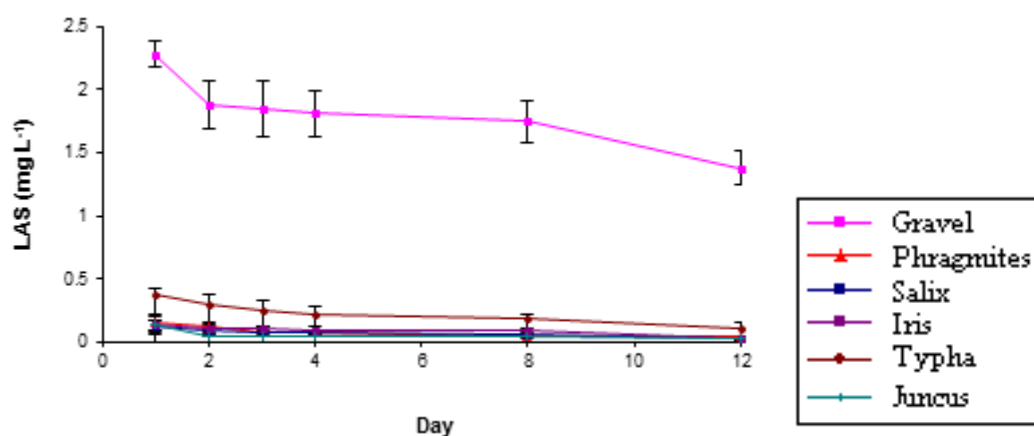


Figure 7. Outflow LAS concentrations planted mesocosms and gravel control. Error bars represent the standard error of the mean ($n = 4$).